UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH ADMINISTRATION

Bureau of Agricultural and Industrial Chemistry
Agricultural Research Center
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TO: G. W. Irving, Jr., Assistant Chief of Bureau, AIC, Washington, D. C.
FROM: M. W. Kies, Biochemist, Biologically Active Compounds Div., ARC, Beltsville, Maryland
SUBJECT: Report of Trip to the University of Wisconsin Centennial Symposium on Plant Growth Substances, Madison, Wisconsin, September 5-7, 1949

Summary: The symposium consisted of a series of general meetings and round table discussions concerned with many phases of the problem of plant growth regulators. Both fundamental research and applications of plant growth hormones to horticultural problems were discussed. Scientists from all sections of the United States and several foreign countries were in attendance. The speakers, representing many widely separated laboratories, were well known for their contributions to this field of research and, in every case, gave excellent presentations of their particular specialties. It was interesting to note that this problem has attracted the attention of many different categories of research workers, namely botanists, plant physiologists and pathologists, biochemists, organic chemists, microbiologists, and physical chemists.

September 5, General Session I.

1. The History and Nature of Plant Growth Hormones, by A. J. Haagen-Smit, California Institute of Technology.

Haagen-Smit and Kogl were the first to isolate a natural plant hormone in crystalline form and to identify the compound so obtained. After giving his definition of auxin (an organic compound which promotes longitudinal plant growth when applied in extremely small amounts) and phytohormone (an active compound produced naturally in higher plants and translocated in the plant tissue), Dr. Haagen-Smit discussed the historical background of their work on auxin a and b and heteroauxin.

Dr. Went developed the first quantitative techniques for the study of plant hormones. His Avena assay method was instrumental in the isolation and identification of the naturally occurring auxins. Since then several other techniques have been utilized, namely, avena coleoptile growth (in which the increase in length is proportional to the log of the concentration of the plant growth regulator) and Went's split pea stem curling method. The bean curvature test, which was used for the extensive surveys carried out at Camp Detrick and also used as the test method in the Bureau of Plant Industry, Soils, and Agricultural Engineering, was not mentioned by the speaker. The need for transportability within the plant

serves to differentiate the Avena test from other plant tests and limits its usefulness for general survey purposes. Haagen-Smit cited the many difficulties inherent in the Avena curvature method but concluded that with sufficient care it can be a reliable test. It is difficult to interpret Avena test results with natural plant extracts because of the presence of growth inhibitors, such as parasorbic acid and anemonin in the extracts.

Auxin a and b were found to be quite unstable, even in the crystalline state, and it was not possible to establish complete proof of structure by synthesis. Ultraviolet light converts auxin a lactone into an inactive "lumi-auxone" by splitting out two molecules of water from the acidic side chain. Auxin a could be oxidized to give auxin a glutaric acid, which has been synthesized.

(There was much conjecture at the meeting as to whether this work could be repeated and confirmed. It is extremely significant that no one, even in Haagen-Smit's own laboratory has been able to find such compounds in samples of corn oil or urine as was originally claimed. A possible explanation is that suxin a and b were actually derivatives of indoleacetic acid and erroneously identified. This is merely conjecture and the problem is still unsolved).

The problem of the identity and function of natural plant growth regulators is extremely complex. Although the preponderance of work has been done on indoleacetic acid as a typical auxin, it is by no means the only naturally-occurring compound known to possess activity. With the split pea stem as test material phenylacetic acid and cis-cinnamic acid have also been found to be active. Van Overbeek has shown with deseeded and derooted avena coleoptiles that the curvature obtained with natural plant growth regulators showed up in less time than curvature induced by indoleacetic acid. Indoleacetaldehyde, which is active, has been found by Larsen to occur naturally. Tryptophan is known to be converted to indoleacetic acid by relatively mild treatment and may account for some of the natural activity reported by various workers. English and Bonner have isolated a wound hormone (traumatic acid) which stimulates tissue growth in the vicinity of injuries. Glutamic acid enhances its activity markedly. (In this connection, it has been observed that arginine, adenine and methionine each enhance indoleacetic acid activity under certain test conditions.)

2. The Synthetic Auxins: Structure and Function, by K. V. Thimann, Harvard University

Factors involved in testing for plant growth regulator activity.

a. Ability of compounds to enter cell and translocate within the plant.
b. Stability to plant enzymes and other plant constituents.
c. Stability to light.
d. Dissociation of hydrogen ion.

These factors may be minimized by using short time tests, floating test material in solution, working in the dark at known pH, and using plant test material low in natural plant growth regulators. He recommended the split pea stem curvature as the test for primary growth effects.

Structural requirements for auxin activity:

a. Unsaturated ring system (cyclohexane acetic acid is inactive). A five-membered ring alone is not sufficient; fusion with a six-membered ring is necessary, although the six-membered ring is itself sufficient.
b. Acetic, propionic or butyric acid side chains or acid derivatives (except that α-naphthylnitromethane possesses slight activity indicating the carboxyl may not be absolutely essential). Salts are less active than the acids; methyl esters and amides have about the same activity as the free acids.
c. Double bond in ring must be adjacent to the side chain.
d. At least one hydrogen is essential on the carbon atom alpha to the ring, therefore, the carboxyl must be separated from the ring by at least one methylene group.
e. Spatial configuration is extremely important. Where cis and trans forms are possible, as in the cinnamic acids, one form is active, the other inactive. In cases of optical isomerism differences as great as thirty-fold have been observed in the activities of the two isomers.
f. Miscellaneous structural requirements: α-naphthalene derivatives are more active than the corresponding β-compounds. The sulfur homolog of phenoxyacetic acid is highly active. A methoxyl on the ring or hydroxyl on the carbon alpha to the ring may prevent activity entirely.

The o- and p-nitrophenoxyacetic acids are inactive whereas the m-derivative is active.

Evening Lectures.

1. Twenty Years Work with Plant Growth Substances, by F. W. Went, California Institute of Technology.

The speaker was educated in Utrecht, Holland in the laboratory of his father who was also a well known plant physiologist. He came to the United States in 1933. F. Darwin in 1880 observed that the phototropic effect on the tips and roots was transmitted to other plant parts. Sachs demonstrated that some material produced in tips of cuttings moved downward and caused rooting at the base. J. Loeb and Fitting both studied the effects of illumination of plants but Fitting so misinterpreted his results that the discovery of plant hormones was held up for several years. Fitting, however, was the first to demonstrate hormonal effect of orchid pollen extracts in vivo. Boysen-Jensen's experiments several years later straightened out the concept of stimuli transmission somewhat but he too failed to understand completely the principle of hormonal control of plant reactions.

Even though much has been done on the extraction of auxins from plant materials and correlation of extraction results with plant phenomena, the problem is still open as to what actually is the relationship between indoleacetic acid (or other hormones) and growth. Went ended his speech with the humorous comment that he felt (after the earlier speeches of Haagen-Smit and Thimann) he had been present at the demotion of auxin from an executive position to an ordinary policing job and this left many positions yet to be filled.

2. Some Practical Applications of Plant Growth Substances, by P. W. Zimmerman, Boyce Thompson Institute for Plant Research.

The herbicidal effect of certain plant growth regulators, such as 2,4-D, is at present the most important application. Second in commercial importance is the use of naphthaleneacetic acid to prevent pre-harvest fruit drop. Other applications include: increase of fruit set, inhibition of potato sprouting in storage, prolonging dormancy of buds during a late frost, inhibition of buds on nursery stock, regulation of the flowering of pineapples (this is the only known instance of the stimulation of flowering by plant growth regulators). The rest of Zimmerman's talk was concerned with the control of the water hyacinth with 2,4-D sprays. He showed several colored slides illustrating the remarkable success of this treatment in clearing the inland water-ways of Louisiana.

September 6 - Round Table Discussion on Role of Growth Substances in Plant Metabolism. Chairman R. H. Burris, University of Wisconsin.

A major problem is to describe in biochemical terms the mode of action of an auxin. The multiplicity of effects described for the various active compounds makes this extremely difficult. We can assume that auxins are involved in certain (unknown) enzyme reactions which together constitute the growth phenomenon. Since such minute quantities are effective it cannot function as an enzyme substrate.

It is possible that a study of the herbicidal action of auxins may yield clues as to their biochemical functions: (a) increased mobilization of carbohydrates, indicating an effect on plant amylases, (b) increased nitrogen content caused by carbohydrate depletion, suggesting study of transaminases and reductive amination reactions, (c) increased respiration at low concentrations and decreased respiration at high concentrations, pointing to the relationship of respiratory enzymes to herbicidal action.

1. Relation of Growth Substances to Enzyme Systems, by K. V. Thimann, Harvard University.

Work in Thimann's laboratory has been directed toward a study of the relation of auxin to the metabolism of the four carbon acids (succinic, malic, and fumaric). They studied isolated plant parts grown under controlled conditions (avena coleoptila cylinders and etiolated pea stem sections). The iodoacetate ion is a powerful inhibitor of the growth of these sections but this inhibition can be overcome by the addition of any of the 4 above mentioned organic acids. During growth reducing sugars disappear and when growth is inhibited by the iodoacetate ion the sugars are used up even faster. Thimann believes that the sugars are being converted to fat.

Conversion of amino acids to protein, which occurs during growth of the plant sections, is enhanced by auxins and inhibited by growth inhibitors, such as arsenite, fluoride and iodoacetate ions.

In the presence of sodium malate, the respiration of the plant sections, as well as growth, is enhanced by auxin. Thimann suggested that succinic dehydrogenase (the "cross roads of carbohydrate metabolism) exists in

conjunction with a natural inhibitor and that indoleacetic acid merely releases the enzyme from the normal state of inhibition.

2. Stimulation of Respiration in Relation to Growth, by G. S. Avery, Jr., Brooklyn Botanical Garden.

Pretreatment of coleoptile sections with indoleacetic acid stimulated subsequently isolated malic and alcohol dehydrogenases enzyme systems. At low concentrations 2,4-D stimulates respiration of pea and avena sections about 20% (peas are more sensitive than avena) but at higher concentrations both are inhibited. Indoleacetic acid and 2,4-D affect growth very nearly the same but 2,4-D is much more effective in stimulating respiration so the two compounds may be affecting different enzyme systems.

At this point, Dr. Robbins of the New York Botanical Gardens engaged Thimann and Avery in a discussion on auxin terminology. Robbins contended that Auxin and indoleacetic acid were not necessarily synonymous and should not be used in this manner. Thimann said auxin is anything which stimulates longitudinal growth; it is a generic term, and therefore could be used correctly as a synonym for indoleacetic acid.

3. Inhibition of Respiration in Relation to Toxicity, by F. G. Smith, Iowa State College.

Indoleacetic acid and 2,4-D were compared with respect to toxicity and respiratory changes. Wheat root elongation is inhibited by indoleacetic acid but respiration is not affected. 2,4-D causes increased utilization of carbohydrate, increased protein synthesis, and increased oxygen uptake (except that the oxygen uptake falls off rapidly after definite symptoms of toxicity are apparent). Aerobic respiration of plants is particularly sensitive to both indoleacetic acid and 2,4-D whereas the anaerobic respiration is relatively insensitive.

Round Table Discussion on the Role of Growth Substances in Vegetative Development.

1. The Growth of Tissues in Culture, by P. R. White, Institute for Cancer Research, Philadelphia.

Indoleacetic and naphthaleneacetic acids stimulate early growth of tissue sections and in large concentrations cause rooting. If still higher concentrations are applied, polarity and organization of tissue are completely lost.

The role of auxins in vegetative development of tissue cultures is still problematical. Gautheret was the first to culture successfully normal plant tissue (i.e. establish a tissue strain which could be subcultured and kept alive indefinitely). He used carrot cambial tissue and found that the critical nutrient was indoleacetic acid. Certain preparations under went "habituation" after several subcultures and developed the ability to continue growing without added indoleacetic acid. The phenemonon is as yet unexplained.

Crown gall tissue, which can be cultured and kept alive relatively easily, contains more indoleacetic acid than the "habituated" tissue which in turn

contains more of the acid than normal carrot tissue. Is there an enzyme present in normal tissue which destroys indoleacetic acid as it is formed? (Went objected to this as a possible explanation; he preferred the explanation that cells might regain ability to form auxin as a result of a reverse mutation such as has been observed in Neurospora.)

2. Factors Influencing Growth of Plant Embryos, by Nancy Kent Ziebur, University of Wisconsin.

In vitro culture of exicsed plant embryos requires water, minerals and sugar but the embryo will not develop if it is too young. Cocoanut milk, malt extract, certain amino acids, indoleacetic acid, etc., aid in growth and differentiation of these "too young" embryos. Certain factors in casein hydrolysates stimulate embryonic growth and at the same time inhibit germination. The germination effect is probably due to the high osmotic pressure.

3. The Formation and Growth of Buds, by F. Skoog, University of Wisconsin.

Plant tissues in submerged cultures form buds unless indoleacetic acid is added. On the other hand, phosphate ion counteracts the indoleacetic acid effect. Adenine promotes initiation of buds on tobacco stem slices grown on agar. Horse radish root cultures form buds easily but indoleacetic acid inhibits this budding completely at certain concentrations.

(I asked Dr. P. R. White if he thought this could be used as an alternative assay method for plant growth regulators similar to indoleacetic acid and he said he believed it could, although he knew of no instance in which it had been used. The disadvantage is that the method would require 10 days rather than the usual 4 to 24 hours required by other methods.)

Skoog suggested that specific organ forming factors do not exist but that specialization in the developing plant is due to an equilibrium of several growth factors. Went stated at this point that he thought it very unkind to say that a specific organ forming factor did not exist merely because it had not been isolated.

4. The Development of Stems and Leaves, by F. W. Went, California Institute of Technology.

Peas germinated in the dark and kept in the dark do not form normal size leaves. It can be shown that the leaf growth factor comes from the cotyledons and light is necessary for its formation. The factor has been named "phyllocaline". (This is an old theory of Went's which seems to have been pretty well invalidated by recent work.) Adenine and hypoxanthine have recently been shown to influence leaf growth.

Stem growth is dependent on the connection between stem and roots. When a plant part, such as the coleoptile, is severed and then regrafted, it will start to grow only when vascular connections are reestablished. Cut off stems can not be forced to grow again (without rooting). An exception to this rule is asparagus stem tips which can be grown artificially. What is this factor which the roots normally supply--a "stem growth factor"? Indoleacetic acid alone is not sufficient to induce this effect.

September 7, Round Table Discussion on the Role of Growth Substances in Reproductive Development.

1. Sex Hormones in Fungi, by J. Raper, University of Chicago.

Raper and his co-workers have studied the chemicals secreted by fungi which regulate development of the sexual organs. Six distinct hormones have been demonstrated, 4 male and 2 female.

Male and female vegetative forms grown in adjacent media (but not actually in contact) secrete unknown substances A and A' which stimulate development of antheridial hyphae in the male; these structures when formed give rise to a second unknown compound, B, which stimulates oogonal initials in the female. Compound C is then secreted by the female form which causes delimitation of antheridia. Compound D in turn causes delimitation of oogonia, etc. This scheme has been worked out by Dr. Raper and his associates by the preparation of cell free extracts which give rise to the sexual differentiation described. Compound A, which has been most extensively studied, has been concentrated so that it can be detected in a dilution of the order of 10^{13}.

2. Growth Regulating Substances in Relation to Reproduction of some Corn Plants, by A. E. Murneek, University of Missouri.

These are two periodic stimulations of growth in corn plants connected with germination which can be correlated with plant growth regulator production. Is a growth regulator actually liberated during germination or is an enzyme liberated which causes formation of the plant growth regulator?

Fruit containing many seeds are more susceptible to the effect of plant growth regulators than single seeded types. In the latter, hormones can only be used to induce fruit thining. Embryonic development is necessary to fruit growth in single seeded species and therefore no parthenocarpic fruit can be formed.

The endosperm functions as a nutritive agent for the embryo since it has been shown that isolated corn embryos require a specific hormone present in the endosperm (not indoleacetic acid) for their development. Mention was made here of the embryo factor discovered by Van Overbeek in coconut milk which is important in the development of orchid embryos.

3. The Induction of Flowering with Plant Extracts, by R. H. Roberts, University of Wisconsin.

The effect of a "crystalline" extract of alfalfa on the flowering of a cockle burr was discussed in some detail. The material is admittedly not homogeneous (may be partly inorganic) and its effect is merely to encourage formation of flower primordia under subminimal light conditions. The mineral salt of a fatty acid is one component but this fraction has not been tested for activity. No further information was available concerning the chemistry of the extract.

This report was disappointing in many respects but the subject has such great fundamental significance (the possible existence of a flower hormone) that it can not be dismissed entirely. The only instance so far reported

of the effect of plant growth regulators on flowering is the induction of flowering in the pineapple by indoleacetic acid and similar compounds.

4. The Role of Growth Substances in Parthenocarpy, by F. G. Gustafson, University of Michigan.

Gustafson in 1916 was the first to produce parthenocarpic fruit by the application of plant growth regulators to the pistil. Phenylacetic, indoleacetic, indolepropionic, indolebutyric acids are all effective in the 0.25 to 2% concentration range. In 1934, Yasuda produced parthenocarpic fruit with natural plant growth regulators. In 1937, Gardner and Marth induced fruit formation in the Ilex with hormone sprays.

5. The Growth Hormone Mechanism in Fruit Development, by R. Muir, University of Iowa.

The auxin production mechanism starts at fertilization, but the style contains 30 times as much plant growth regulator as the pollen and the ovary 100 times as much, so the pollen must contribute something other than extra hormones. Muir suggested that auxin in the ovary may be formed from tryptophan and pollen may contribute (enzyme?) to the formation of tryptophan.

6. The Role of Growth Substances in Fruit Setting, by S. H. Wittwer, Michigan State College.

This was one of the best presented speeches at the symposium; it differed from most of the others in that it was concerned with the application of plant growth regulators to tomato production. Some tomato varieties are affected adversely by low light intensities and the blossoms drop off before fruit set but this can be prevented by application of plant hormones. The parthenocarpic fruit ripen earlier and thus increase the early yield when prices are still high. Total yields for the season are not changed appreciably. Later tomato varieties usually respond to treatment by an increase in total yield. Plant growth regulators, in the series reported, also reduced blossom end rot, contrary to other reports.

General Session--Roles of Vitamins and Amino Acids as Growth Factors for Plants.

1. Growth Factors in Bacterial Nutrition, by E. E. Snell, University of Wisconsin.

Amino acids and vitamins as growth factors for bacteria vary from species to species. Miscellaneous factors also found to be essential for bacterial growth include: conjugated vitamins, fatty acids, purines and pyimidines, nucleosides and nucleotides, asparagine and glutamine, hemin, putrescine, etc., all of which are common metabolic products of living forms. Bacteria have, however, lost the ability to synthesize them. Failure to synthesize growth factors may be one of two things: the conversion of A to B may be blocked by lack of the necessary enzyme (genetic block) or by inhibition of the enzyme by another factor present in the medium. For example, E. coli requires phenylalanine only if tyrosine is added to the medium. If certain metabolites are inhibitory, their antagonists appear to support growth. This fact should be considered in interpreting results obtained when studying bacterial growth factors.

2. Vitamin and Amino Acid Requirements for Growth of Higher Plants, by W. J. Robbins, New York Botanical Garden.

Higher plants require no vitamins; they appear to synthesize adequate amounts. (Went says certain plants require lots of mulch because they cannot supply their own vitamin B, entirely.)

Isolated plant parts do require vitamins; excised roots require several different ones, including thiamine, niacin and pyridoxine. Indole acetic acid is important for growth of stem sections but not for roots. Embryos freed of endosperm need biotin and niacin as well as other unknown factors.

September 8 - Conferences with the following:

Dr. F. T. Addicott, Department of Botany, University of California at Los Angeles.

(Dr. Addicott was formerly employed on the Guayule project at the Salinas Laboratory) We discussed his work on foliar abscission. They have developed methods for studying this phenomenon in vitro with stem and leaf preparations. Some of their techniques might be applicable to a study of natural plant growth regulators. He stated that they have demonstrated a compound present in bean stems (not an enzyme) which destroys indole-acetic acid.

Dr. W. H. Minshall, Department of Botany, Central Experimental Farm, Ottowa.

They are working on weed killers, studying their effects on plant respiration and photosynthesis by means of infrared spectrographic analysis of CO_2 and H_2O. Their instrument has been designed to detect and measure only these two compounds in extremely small concentrations.

Dr. P. R. White, Cancer Research Institute, Philadelphia.

We discussed techniques of plant tissue culture. White's book is a classic on this subject and was quoted by several speakers during the symposium. He suggested the use of the rapidly growing meristematic tissue of seedling blue grass as a possible test material for some of our plant extract. This tissue might also be useful for experiments using a Warburg respiratory apparatus.

Dr. H. R. Burris, Department of Biochemistry, University of Wisconsin.

I visited his laboratory and discussed their recent work on the effect of plant growth regulators on plant tissue respiration. He stated that they had so far been unable to detect an effect of these compounds on respiration or isolated enzyme systems other than marked inhibition at high (definitely non-physiological) concentrations. He suggested the use of an inexpensive hand-operated microtome for preparation of tissue slices for respiration studies.

Dr. F. Skoog, Department of Botany, University of Wisconsin.

He was busy finishing up details connected with the publication of the papers presented at the Symposium and was not able to spend any time with me. However, one of his graduate students, Mr. Wiggin, showed us around the

laboratory and discussed their work. They are studying the culture of tobacco stem slices and carrot cambial tissue slices. With proper nutrient agar (White's nutrient formula) containing added adenine, indoleacetic acid and phosphate, they are able to grow in Erlenmeyer flasks a small but complete plant, i.e., roots, shoots, and leaves. They believe that a phosphorylating enzyme system is important in shoot development but Skoog had said during his discussion earlier that constituents of ATP were not as effective adenine in inducing shoot growth. They have not tried ATP itself. Mr. Wiggin described in detail the Avena curvature assay method for determining plant growth regulators. He had found this method very difficult at first but after considerable practice found that it gave reasonably consistent results. (This was the opinion generally expressed by others at the symposium with whom I discussed this method.) The procedure is simple and requires no specialized equipment other than very accurate constant temperature and humidity control.

The papers presented at the Symposium will all be published in a monograph by the University of Wisconsin in the near future.

Conclusions and Recommendations:

The Symposium served to acquaint us with the historical background and recent developments in plant-growth regulator research. This orientation in a field which is relatively new to our group was more effective than many weeks spent in library research. Although most of the material presented had already been published it was discussed in much greater detail at the Symposium. Valuable personal contacts were made with leading plant physiologists and biochemists who are actively engaged in plant growth regulator research.

Points of technique which may prove useful in our project were discussed with others attending the Symposium. Of particular interest was the possibility of using seedling blue grass as an alternate assay method, or as experimental material for studying the mode of action of natural plant growth regulators.

The details of this meeting have been reviewed with Dr. J. W. Mitchell, plant physiologist, Bureau of Plant Industry, Soils, and Agricultural Engineering, who conducts the plant-growth regulator assays under our cooperative project.

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